The effect of ascorbic acid on glucose respiration of *Saccharomyces cerevisiae*

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Introduction

Saccharomyces cerevisiae (commonly known as baker's yeast) is an opportunistic fungus widely used to ferment human edibles, such as bread and beer (Maicas et al., 2020). As an opportunistic pathogen, *S. cerevisiae* exhibits the potential to cause illness in immunocompromised individuals. However, the nature of virulent *S. cerevisiae* strains remains less known because the fungi are unlikely to cause infection.

Since the 1990s, the number of fungal infections caused by *S.cerevisiae* has increased while exhibiting risk factors akin to fungi responsible for candidemia (Enache-Angoulvant et al., 2005). Severe cases of *S.cerevisiae* infections have been primarily found in critically ill patients suffering from immunocompromising underlying conditions such as cancer, HIV infections, and neutropenia (Muñoz et al., 2005). With the emergence of COVID-19 in 2020, *S.cerevisiae* has also been reported as a co-infectious pathogen in intensive care units of SARS-2 infected patients' bloodstream (Ventoulis et al., 2020).

Biofilm, an organic substance created by cells, plays a vital role in the virulent spread of *S.cerevisiae* infections. While there has been limited research done focusing on the significance of biofilms in eukaryotes in contrast to prokaryotes, confocal laser scanning microscopy shows that the biofilm structure of *S.cerevisiae* is homologous to other infectious yeasts such as *Candida albicans* and *Candida glabrata* (Bojsen et al., 2014). *C.albicans* and *S.cerevisiae* are also known to be phylogenetically close to each other (Pérez-Torrado et al., 2016). This finding means much of the information surrounding *C. albicans*, a fungus that is more prevalent and studied, applies to this study. It has been reported that biofilm promotes resistance to antimicrobials by 10-1000 times in *C. albicans* (Douglas, 2003). Biofilm has also been suggested to be relevant in preventing an immune response in a host's body. As opposed to planktonic *C.albicans* cells, leukocytes fail to phagocytose biofilm dispersed cells (Chandra et al., 2007).

While these examples demonstrate the substances' significance in defense of the cell, biofilms are also part of the arsenal the fungus uses to cause infections. In contrast to planktonic variants of *C.albicans*, biofilm dispersed yeast cells exhibit a greater tendency to adhere to surfaces and are more virulent by having direct access to the bloodstream (Uppuluri et al., 2018). The bloodstream's availability to the fungus unleashes the potential of metastatic infections within the organs of the host (Uppuluri et al., 2010). Most notably, biofilm production allows *C.albicans* to take residence within the mucosal tissue of the host's oral cavities, leading to oral infections (Tsui et al., 2016). *C.albicans* has also been reported to reside within the vagina, causing vaginitis-related infections (Sobel et al., 1984).

Within *S.cerevisiae*, the efflux pump also serves as a crucial biological apparatus to cause infections. This pump is a protein located within the cell membrane and is responsible for regulating homeostasis within the cell by diffusing excess substances towards the environment (Pearson et al., 1999). The overexpression of genes encoding for this protein is becoming a prevalent concern across the medical community, as it contributes to growing antimicrobial resistance in microorganisms (Willers et al., 2017). This concern applies towards *S.cerevisiae* because the gene encoding for the multidrug-efflux pump known as PDR5 is a homologue to the CDR1 gene found within *C.albicans* (Nakamura et al., 2001). The CDR1 gene is notorious for encoding an energy-dependent multidrug efflux pump responsible for the decrease in susceptibility to azole drug derivatives (Sanglard et al., 1999). In another study, continuous upregulation of the efflux pump has been sufficient to induce an overall resistance towards all azole derivatives (Pfaller, 2012).

While antifungal medications have been deployed against the fungus, the tolerance of *S.cerevisiae* to numerous antifungals are poorly understood (Zerva et al, 1996). Despite *S.cerevisiae's* known resistance to every azole derivative, it has shown consistent susceptibility to the common drug therapy of amphotericin B and flucytosine (Muñoz et al.,

2005). Yet there is a risk to use these two drug therapies frequently due to concerns involving the toxic reactions it may have on patients (Stamm et al., 1987). As of 2017, new antifungals were produced to add to the variety of treatments available, though many possessed chronic or acute negative side effects when administered into patients (Campoy & Adrio, 2017). Furthermore, with the similarity between *C.albicans* and *S.cerevisiae*, it is likely that it shares an identical issue in the redundant use of molecular targets in which the former has begun to grow resistance to. Both of these variables limit the deployment and effectiveness of new treatments and calls for new approaches to combat *S. cerevisiae*. (Sangamwar et al., 2008).

With the current growing antimicrobial resistance to yeast infections and the limitations in current treatment, identifying a new molecular target is as prevalent as ever. Cellular respiration is a process that cells undergo to metabolize organic molecules to an energy molecule known as adenosine triphosphate (ATP). Although lipids and proteins can be used in respiration, glucose is the organic molecule mainly used in the process. In glucose respiration, glucose molecules are converted into pyruvate through a process known as glycolysis (Caballero et al., 2016). *S.cerevisiae* is a facultative anaerobe meaning that it has the option to respirate in the absence or presence of oxygen (Krantz et al., 2004). Under the presence of oxygen, pyruvate is converted into several organic intermediate molecules—eventually producing 30-32 ATP (Berg et al., 2002). One byproduct of this process is the release of carbon dioxide (CO_2) into the atmosphere. Alternatively, in the absence of oxygen, pyruvate is converted into biofuel, with 2 ATPs and CO_2 being produced as a result of anaerobic respiration (Kelly et al., 2001).

The mitochondria's significance as a potential target for new drugs stems from the aforementioned virulent factors' (biofilm and efflux pump) dependency on the organelle and its function. As the efflux pump protein encoded by PDR5 depends on ATP to function, disrupting the production of the energy molecule may inhibit the protein's effectiveness

(Decottignies, 1994). A study explored this concept by applying cyanide to the mitochondria of *C. albicans*. Affected fungal cells exhibited increased susceptibility to fluconazole, an azole derivative normally ejected by the efflux pump (Yan et al., 2009). However, due to cyanide's toxicity towards humans, its practical application as a remedy remains limited (Holland, 1986). Mitochondrial respiration also plays a significant role in biofilm formation with specific proteins found within the mitochondria being responsible for biofilm maturation (Calderone et al., 2015). Anaerobic respiration has also even been shown to increase the sturdiness of biofilms, indicating its importance in infections (Yoon et al., 2011). This relationship between biofilm production and mitochondria has been proposed as a possible target for future antifungals in recent studies (Xue et al., 2019). However, exploration of this implication remains limited in scope.

Ascorbic acid is a cofactor responsible for glucose respiration and resistance against infectious diseases in humans (National Center for Biotechnology Information, 2021). Previous literature of its effects on *S.cerevisiae* indicates that DNA cloned recombinant fungus with the ability to biosynthesize ascorbic acid demonstrated increased anaerobic respiration performance (Branduardi et al., 2007). However, these findings were done through intracellular modification of the cell, thus harmful doses of ascorbic acid could be obstructed through gene regulation— and is a variable the research failed to address (Hahn & Young, 2011). Extracellular insertion of ascorbic acid into *S.cerevisiae* has not been researched, but, in other eukaryotes it has been correlated with mitochondrial impairment (Bakalova et al., 2020). In 2010, one study published in the National Library of Medicine discussed ascorbic acid's potential to inhibit glucose respiration in rat adipocytes. Throughout the experiment, adipocytes were incubated in a culture medium containing a presence or absence of 1.6 nM of insulin, and ascorbic acid from a factor of 5-1000 µM. The results concluded that ascorbic acid affected the respiration of insulin-depleted culture medium

(Garcia-Diaz et al., 2010). While this study suggests a relationship between respiration and ascorbic acid exists, it does not provide much insight on the performance of *S.cerevisiae* due to the major differences in respiration pathways as a result of divergent evolution that spans more than 1600 million years (Wang et al., 1999). Furthermore, the study also included insulin as an additional independent variable, which is irrelevant to the present study.

In a similar vein to the above findings, Avci et al. (2016) investigated respiration changes in *C. albicans* when administering 90 mM of ascorbic acid. The investigators grew *C. albicans* in either dextrose or phosphate-buffered saline growth mediums and monitored their growth by measuring the concentration of mitochondrial NADH produced. The authors concluded that ascorbic acid in this concentration was sufficient to inhibit mitochondrial respiration and induce cell death. This finding applies better to *S.cerevisiae* than those published by Garcia-Diaz et al. because the two fungi share closer evolutionary history and genetics. Despite their similarities, *S.cerevisiae* lacks NADH complex I proteins found in *C.albican*'s mitochondria. (Sun et al., 2019). As complex I is vital in the production of NADH, the absence of complex I in *S.cerevisiae* means the physiological effects that ascorbic may have on baker yeast's respiration remains unknown (Sharma et al., 2009). Thus, this experiment aimed to determine the effects of varying ascorbic acid concentrations towards the cellular respiration of *S. cerevisiae*. The results collected from this experiment will yield insight in generating new implications and directions for antifungal medication towards virulent strains of *S. cerevisiae*.

Methods

As this study imposed a possible treatment towards an experimental unit, and documented empirical evidence, the methodology followed a scientific experimental design with the objective of discovering the effects of varying ascorbic acid concentrations towards the respiration of *S.cerevisiae*. *S.cerevisiae* cultures were groomed in petri dishes and separated

into 3 groups of 2 depending on whether they were the experimental or positive or negative control group. One out of the two yeast cultures for each group represented the operation under aerobic respiration, whereas the other half performed anaerobic respiration. Both types of respiration were evaluated in this experiment to specifically analyze how these two types of respiration fare with the addition of ascorbic acid from a factor of 0.5 to 1.5 mL. Quantitative data from the concentration of carbon dioxide present, and images at a microscopic level assists in creating novel conclusions for the experiment's purpose.

Rationale and Hypotheses

It was hypothesized the following effects would occur in this experiment: (1) If ascorbic acid is inserted into a culture of *S.cerevisiae* then the respiration rate will decrease; (2) If cellular respiration rate continues to decrease then the cell will lyse. Based on Avci et al.'s findings on *C.albicans'* exposure to ascorbic acid, the former predicts ascorbic acid is the underlying mechanism for a decrease in respiration, whereas the latter predicts that the lack of ATP being produced from decreased respiration rate will prevent upregulation of intracellular functions. *S.cerevisiae* strains purchased commercially for this experiment were rationalised as an approximate estimate for the variants responsible for clinical infections. The reason behind this was not only due to the infeasibility of obtaining infectious variations of the fungus, but also because they are in the same species and still contain near-identical genes.

Biosafety Regulations

Due to the experiment involving the incubation of microorganisms in a house setting, biosafety must be considered for the safety of the residents. According to the Laboratory Biosafety Manual published by the World Health Organisation, biosafety regulation is determined by the likeliness for the organism in question to be a pathogen. The Hong Kong University Biological Safety Policy and Guidance Policy deems *S.cerevisiae* to be a part of

risk group 1 (no or low individual community risk), therefore with its unlikeliness to be a pathogen, biosafety level 1 protocols was deemed to be the most appropriate for this experiment (Hong Kong University Safety Office, 2019).

Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

Table 1. *Relation of risk groups to biosafety levels, practices, and equipment. BSC, biological safety cabinet; GMT, good microbiological techniques. Adapted from* World Health Organisation (2004, pp. 2).

Biosafety Level 1 was strictly practiced by having the experiment be performed on a spare flat table. A hand sanitizer spray was used once upon entering the workbench and exiting it, and gloves were worn on site to demote direct skin contact with the fungal culture cells. The surrounding area was sprayed with the sanitiser after completion of each trial in prevention of eliminating spores that escaped through the air due to the opening of the agar plate's lid to measure respiration. Upon finishing the experiment, used agar plates were immediately washed-out with sodium hypochlorite (bleach) and were immediately disposed of in a plastic bag alongside other materials used throughout the experiment. The exception to

this was the CO_2 detector which underwent exposure to not only hand sanitizer sprays but with wet tissue. After the final trial, the detector underwent the previous procedures mentioned before being placed in a plastic bag and was exposed to constant UV rays through the sun for 24 hours. Only afterwards was it considered decontaminated.

Harvesting the Fungus

The experiment extracted from a sample pool of 9 baker's yeast bags (one for each trial). For a single trial, a bag of yeast was poured into a collection tube, and exposed to 2.5 mL of warm water. The tube was then shaken by hand for approximately 5 seconds. Yeast samples were then extracted through sterilised Q-tips and swabbed onto 6 different petri dishes, containing either sabouraud dextrose agar (SDA) or no media. SDA was used as the growth medium for both the positive control and experimental group culture, while no media was used to facilitate the growth of the negative control group in the petri dish. SDA was the most appropriate growth medium for this experiment, because it is composed of dextrose, a monohydrate variant of glucose; which was the input of respiration this experiment was measuring (National Center for Biotechnology Information, 2021). The stroking pattern followed a "zig-zag" pattern horizontally across the petri-dish before the dish was rotated approximately 120 degrees and the same pattern occurred two more times. Incubation time lasted for approximately 24 hours and under a temperature of 20.0-22.2℃.

Addition of Ascorbic Acid

A tablet containing 25 g of ascorbic acid (*s*) purchased from a pharmacy was first stirred and diluted in 300 mL of distilled water under a temperature of 16℃. The surrounding solution was bright orange in color. Then, 0.5 (± 0.25) mL of diluted ascorbic acid (*aq*) was later extracted through a pipette and inserted into the yeast cultures. The surrounding areas turned opaque and grey in color after the insertion of the acid. This procedure repeated three times for each dosage factor, as subsequent variations of this experiment increased ascorbic

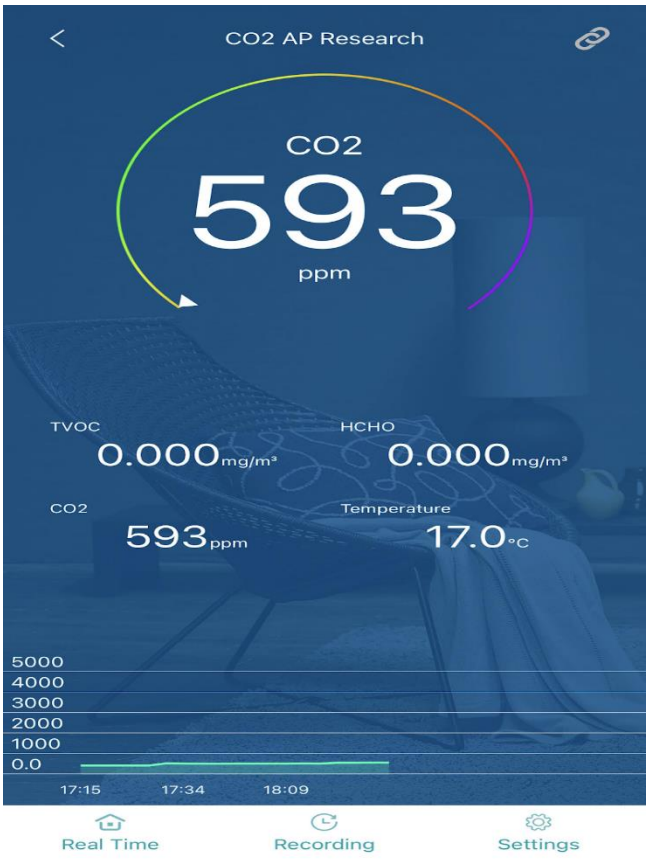
acid concentration by 0.5 mL. For example, yeast cultures stemming from the 3rd experiment would have been exposed to 1.5 mL. Throughout the trial runs, randomly selected colonies for dosage were done by another individual to minimise unconscious biased results. Only after the collection of the respiration rate data from each of the 6 yeast cultures was it revealed which data corresponds to which yeast culture.

Calculating Respiration

To quantify the rate of respiration in the yeast samples, carbon dioxide concentration was chosen as an ideal form of cellular respiration. This method was chosen, because changes in CO_2 atmospheric concentration could be noticed and quantitatively measured with ease using a CO_2 detector (Massaroni et al., 2019). Furthermore, as this experiment was performed at home, it lacked the resources to analyze the effects of respiration under a molecular level (e.g., concentration of respiration coenzymes). CO_2 detectors were vastly more commercially and financially available in contrast to the briefly discussed alternative.

The CO_2 detector model used in this experiment was the WP6003 Bluetooth APP Air Quality Detector and the detector's data was displayed on the XiaoMei Smart app through bluetooth. Respiration rate in this experiment was measured in the concentration of carbon dioxide for every 5 minutes, and for each yeast culture, the detector was first calibrated to measure the initial concentration of CO_2 (ppm) present throughout the household. Therefore, changes in the initial CO_2 concentration when measuring each petri dish is a result of the addition of CO_2 from respiration output.

Picture 1. *Sample screenshot of CO_2 detector and XiaoMei Smart app used to record data. TVOC, total volatile organic compounds; HCHO, formaldehyde; ppm, parts per million.*



Measuring Anaerobic Respiration

Anaerobic respiration yeast cultures had the petri dish's lid be kept on top. This served to stimulate an environment in which oxygen count is negligible or nonexistent in order for the cell to start utilising anaerobic respiration. Once every 5 minutes, the lid was removed momentarily and CO_2 concentration was measured before the lid was placed back on top. This process repeated 5 times for each individual trial.

Measuring Aerobic Respiration

Aerobic respiration required the presence of oxygen for the process to begin. Therefore, groomed yeast cultures that represented this type of respiration throughout the experiment had its lid removed once ascorbic acid was inserted into the experimental group.

Once each trial was over, the cover was immediately placed back on top of the petri dish, and the surrounding area was sprayed with hand sanitizer spray as mentioned above.

Observation of Yeast Under Light Microscope

Unfortunately, the light microscope used during the experiment was damaged - therefore, this experiment was only able to salvage qualitative data from the third trial of each dosage factor. Yeasts after the 5th recording of respiration data followed a wet mounting procedure. *S. cerevisiae* from each group with the exception of the negative control groups were swabbed with a sterilised q-tip onto a microscope slide that contained a 0.5 mL drop of distilled water. In the experimental groups, the sterilised q-tips specifically targeted the opaque regions of the culture medium. Each slide was stained with iodine, and observed under a magnification power of 1200X. The aforementioned staining procedure was replicated for each subsequent trial, and pictures were taken through an iPhone XS Max's camera lens. The pictures taken serves as qualitative data to gather more insight of how ascorbic acid affected the fungus' respiration rate on a microscopic scale and to test whether it induced cell death.

Results

Table 2. Raw data of CO_2 concentration throughout the 25 minutes observation

Cultures	Initial CO_2 Concentration (ppm)	5 minutes CO_2 Concentration (ppm)	10 minutes CO_2 Concentration (ppm)	15 minutes CO_2 Concentration (ppm)	20 minutes CO_2 Concentration (ppm)	25 minutes CO_2 Concentration (ppm)
0.5 mL (Trial 1)						
Aerobic Experimental Group	103	126	483	497	199	212
Anaerobic Experimental Group	211	218	80	114	231	398
Aerobic Positive Control	54	203	138	114	164	75

Anaerobic Positive Control	61	155	83	92	265	120
Aerobic Negative Control	6	0	0	0	0	0
Anaerobic Negative Control	2	0	0	0	0	0
0.5 mL (Trial 2)						
Aerobic Experimental Group	172	548	414	117	611	226
Anaerobic Experimental Group	6	50	21	170	397	338
Aerobic Positive Control	124	330	239	157	127	158
Anaerobic Positive Control	5	8	350	62	121	27
Aerobic Negative Control	1	3	2	0	0	0
Anaerobic Negative Control	0	1	3	0	4	0
0.5 mL (Trial 3)						
Aerobic Experimental Group	5	47	28	33	131	1
Anaerobic Experimental Group	1	2	0	0	1	16
Aerobic Positive Control	66	49	47	31	32	60
Anaerobic Positive Control	6	6	8	29	7	7
Aerobic Negative Control	1	0	0	0	0	0

Anaerobic Negative Control	0	0	6	0	0	6
1 mL (Trial 1)						
Aerobic Experiment	16	7	222	186	131	180
Anaerobic Experiment	166	25	186	147	114	69
Aerobic Positive Control	103	300	267	249	188	117
Anaerobic Positive Control	75	131	11	15	59	7
Aerobic Negative Control	0	0	0	0	0	0
Anaerobic Negative Control	0	0	0	2	0	0
1 mL (Trial 2)						
Aerobic Experiment	95	47	162	91	70	316
Anaerobic Experiment	37	460	182	140	94	891
Aerobic Positive Control	81	440	230	148	466	191
Anaerobic Positive Control	97	60	11	255	146	170
Aerobic Negative Control	2	14	2	6	0	2
Anaerobic Negative Control	7	0	0	2	5	9
1 mL (Trial 3)						
Aerobic Experiment	4	61	41	93	73	28

Anaerobic Experiment	16	2	7	7	6	2
Aerobic Positive Control	50	14	41	5	176	63
Anaerobic Positive Control	9	2	3	4	9	15
Aerobic Negative Control	0	0	0	3	0	0
Anaerobic Negative Control	0	49	0	0	0	0
1.5 mL (Trial 1)						
Aerobic Experimental Group	42	33	59	162	130	131
Anaerobic Experimental Group	62	173	88	10	0	120
Aerobic Positive Control	186	152	165	213	158	347
Anaerobic Positive Control	201	105	195	83	63	202
Aerobic Negative Control	0	0	0	0	0	0
Anaerobic Negative Control	0	0	0	0	0	0
1.5 mL (Trial 2)						
Aerobic Experimental Group	157	36	80	117	29	79
Anaerobic Experimental Group	53	57	214	107	40	29
Aerobic Positive Control	311	214	171	71	53	118

Anaerobic Positive Control	60	346	16	25	55	486
Aerobic Negative Control	0	0	0	0	0	0
Anaerobic Negative Control	0	0	0	0	0	0
1.5 mL (Trial 3)						
Aerobic Experimental Group	21	2	9	22	4	7
Anaerobic Experimental Group	5	50	7	18	10	6
Aerobic Positive Control	20	30	21	66	29	71
Anaerobic Positive Control	5	30	22	20	11	17
Aerobic Negative Control	0	0	0	0	6	0
Anaerobic Negative Control	0	0	0	0	0	0

Table 2. *This table shows the change in CO_2 concentration within each yeast culture using the unit measurement of ppm over the 25 minute observations between the control yeast cultures and yeasts exposed to 25g of ascorbic acid ranging from 0.5 mL to 1.5 mL.*

Calculations

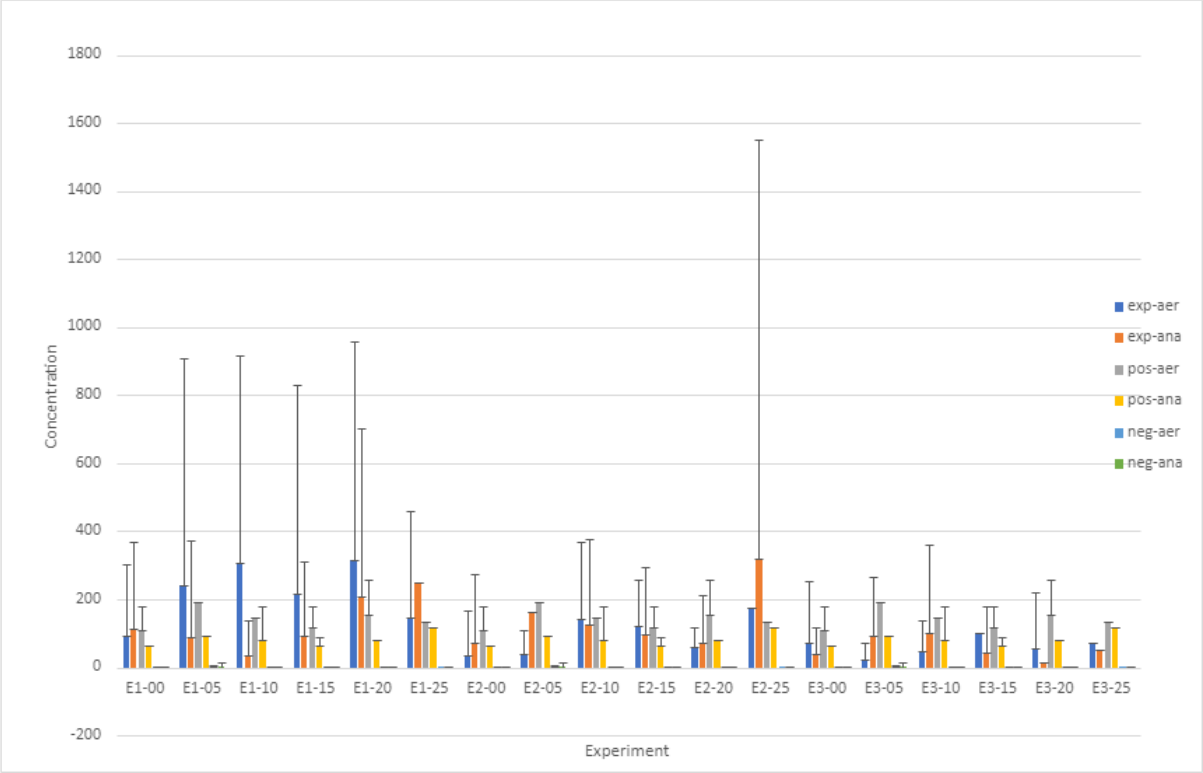
To test the statistical significance of the collected data, an initial assumption was made that the data followed a normal distribution—as the sample size failed to follow the central limit theorem (number of trials>30). This assumption allowed the data to be put through hypothesis testing and analyzed of its variances (ANOVA). A factorial ANOVA was deemed the most fit for this type of data analysis, because there are two independent variables (6 different of *S. cerevisiae* culture and 4 dosage factors) and one dependent variable (CO_2 concentration). The margin of error (MOE) for each point estimate sample mean was calculated under a 95% confidence level using the equation: Margin of Error = Critical Value Standard Error— in which the chosen critical value was a t-score. Unfortunately, as each experimental factor was only tested three times respectively, the range from the data collected was not sufficient enough to determine outliers. Negative control groups were also excluded from this calculation, because mathematically, negative results (getting a 0) would be deemed an outlier using the following equation when it is in fact the purpose of a negative control. The equation to determine outliers was done only towards the positive control groups through the computation of the interquartile range and the numerical threshold using the equation: Lower Outlier Q1-1.5(Q3-Q1)and Higher Outlier Q3+1.5(Q3-Q1).

Table 3. Factorial ANOVA Test

	SS	df	MSQ	F	P(>F)
Yeast Culture	1040955	5	208191	18.102	5.48e-15
Ascorbic Acid Concentration	194848	17	11462	0.997	0.463
Yeast and ascorbic acid interaction	875114	85	10925	0.895	0.719
Error	2484231	216	11501		

Table 3. *This table represents the two-level ANOVA test when the calculated threshold is the following for 3 null hypotheses for the experiment's data to be statistically significant; (1) Yeasts average CO_2 concentration are the same when p>0.001; (2) Average ascorbic acid concentrations are all equal when p>1; (3) There is no interaction between ascorbic acid and yeasts when p>0.1. SS, sum of squares; df, degrees of freedom; MSQ, mean square.*

Graph 1. Average change in CO_2 concentration based on time and dosage factors



Graph 1. *This graph represents the average CO_2 concentration calculated and is separated based on time of measurement and dosage factors. E represents the dosage factors (E1 = 0.5 mL, E3 = 1.5 mL) and the two digits following the dash, demonstrates time (e.g -00 = 0 minutes, -20 = 20 minutes).*

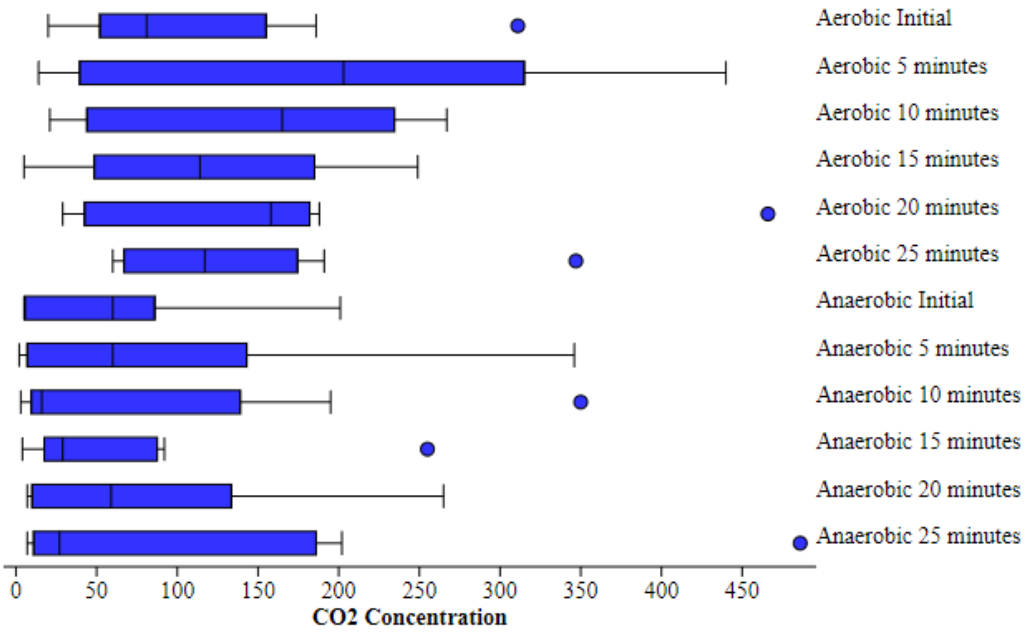
Table 4. Summary table of CO_2 mean respiration rate in varying cultures

	Initial	MOE	5 minutes	MOE	10 minutes	MOE	15 minutes	MOE	20 minutes	MOE	25 minutes	MOE
Average Experimental Aerobic 0.5 mL	93.3	208.48	240.3	669.18	308.3	609.18	215.7	614.21	313.7	645.28	146.3	313.17
Average Experimental Anaerobic 0.5 mL	113.7	255.60	90.0	281.76	33.7	103.06	94.7	215.22	209.7	494.03	250.7	510.34
Average Experimental Aerobic 1 mL	33.3	132.70	38.3	69.58	141.7	229.05	123.3	134.81	58.0	58.18	174.7	357.92
Average Experimental Anaerobic 1 mL	73.0	201.77	162.3	641.06	125.0	253.92	98.0	195.96	71.3	142.73	320.7	1229.88
Average Experimental Aerobic 1.5 mL	73.3	181.89	23.7	46.73	49.3	90.62	100.3	177.54	54.3	165.75	72.3	154.69
Average Experimental Anaerobic 1.5 mL	40.0	76.12	93.3	171.60	103.0	259.13	45.0	133.76	16.7	51.71	51.7	149.79
Average Positive Aerobic	110.53	68.76	192.43	112.94	146.53	70.79	117.13	63.07	154.80	101.88	133.37	70.70

Average Positive Anaerobic	64.3	51.35	93.633	90.14	82.0	96.0	65.0	25.48	81.76	59.06	116.77	128.54
Average Negative Aerobic	1.1	1.50	1.8	3.57	0.44	0.67	1	1.64	0.66	1.55	0.222	0.51
Average Negative Anaerobic	1.125	1.80	0.125	12.52	1.125	1.64	0.5	0.51	1.125	1.55	1.88	2.61

Table 4. *This table represents the average respiration rate throughout the 25 minutes of observations based on the different types of yeast cultures. MOE, Margin of Error.*

Graph 2. Positive controls CO_2 concentration boxplot and outliers



Graph 2. *The multi-group boxplot represents the CO_2 concentration collected throughout the experiment in the different intervals. It is separated into groups representing the type of respiration and time they were collected in. Outliers are represented using blue dots.*

Figure 1. Trial 3 yeast cultures in 0.5 mL experiment

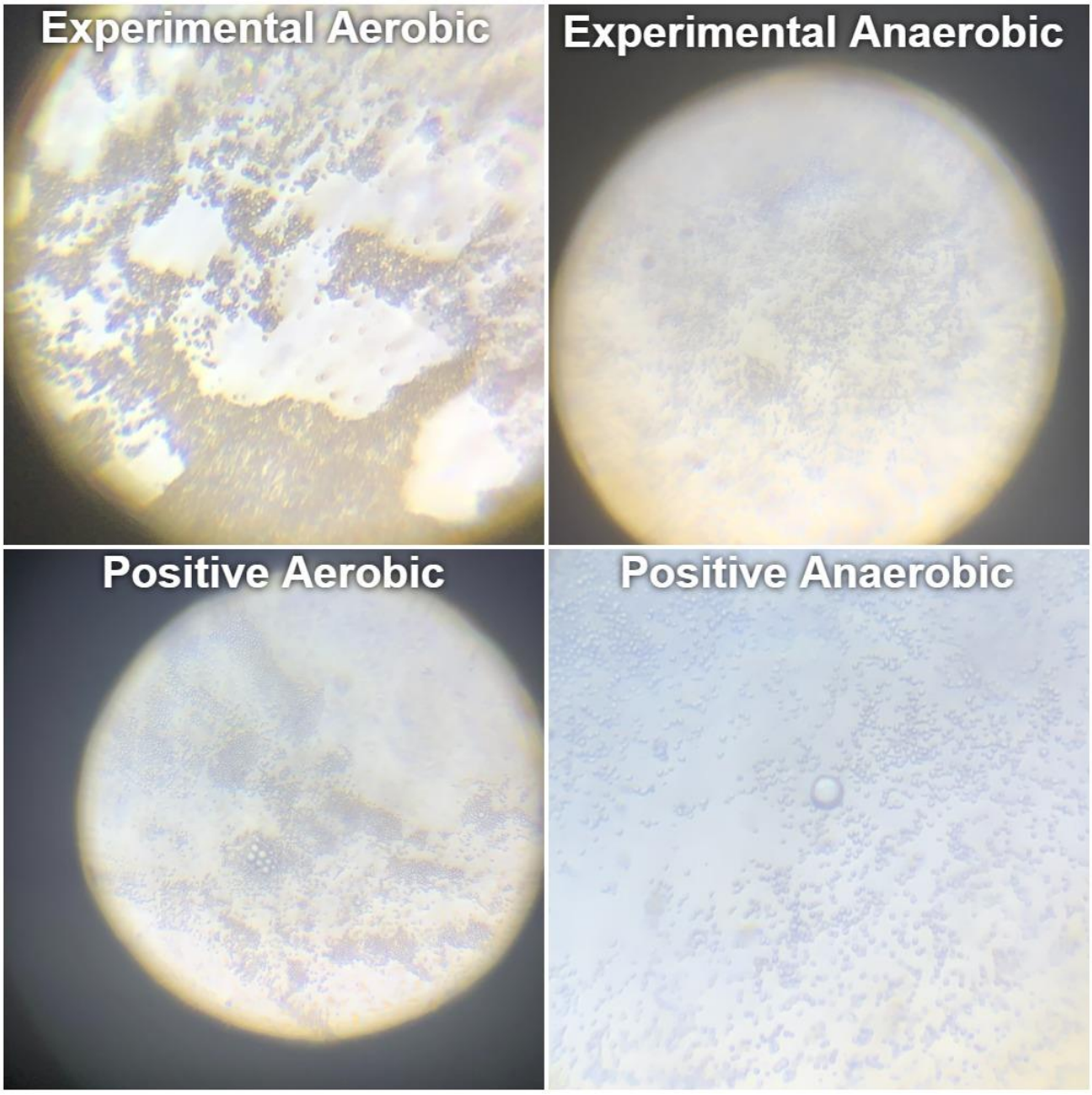


Figure 1. *The darkish brown "dots" on the imaging are yeast cells.*

Figure 2. Trial 3 yeast cultures in 1 mL experiment

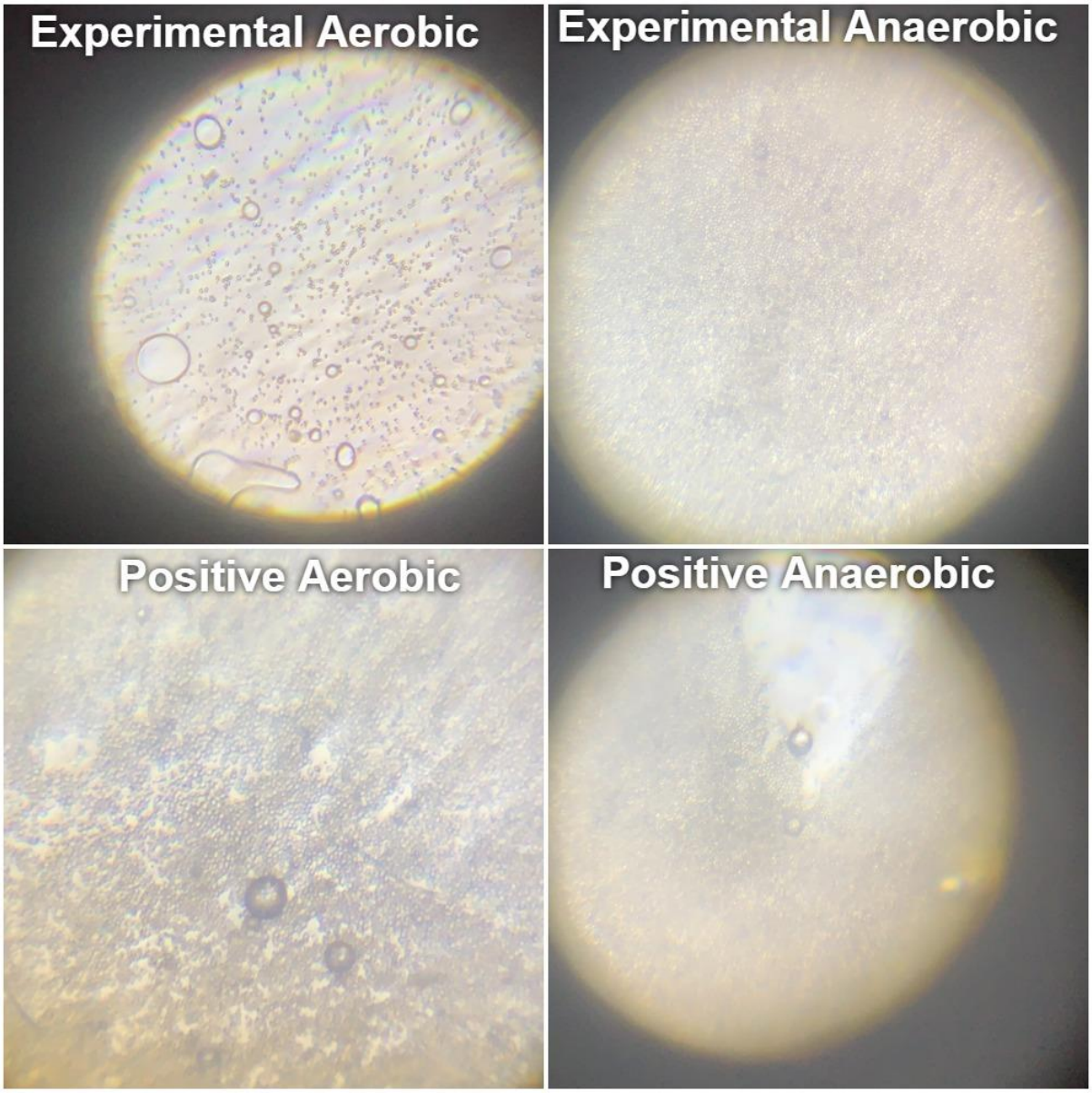


Figure 2. *Water bubbles and foreign debris are found on the top left and right as well as the center bottom of the experimental group measuring aerobic respiration. The positive controls' debris are found in the center of each image—and are circlish black in color.*

Figure 3. Trial 3 yeast cultures in 1.5 mL experiment

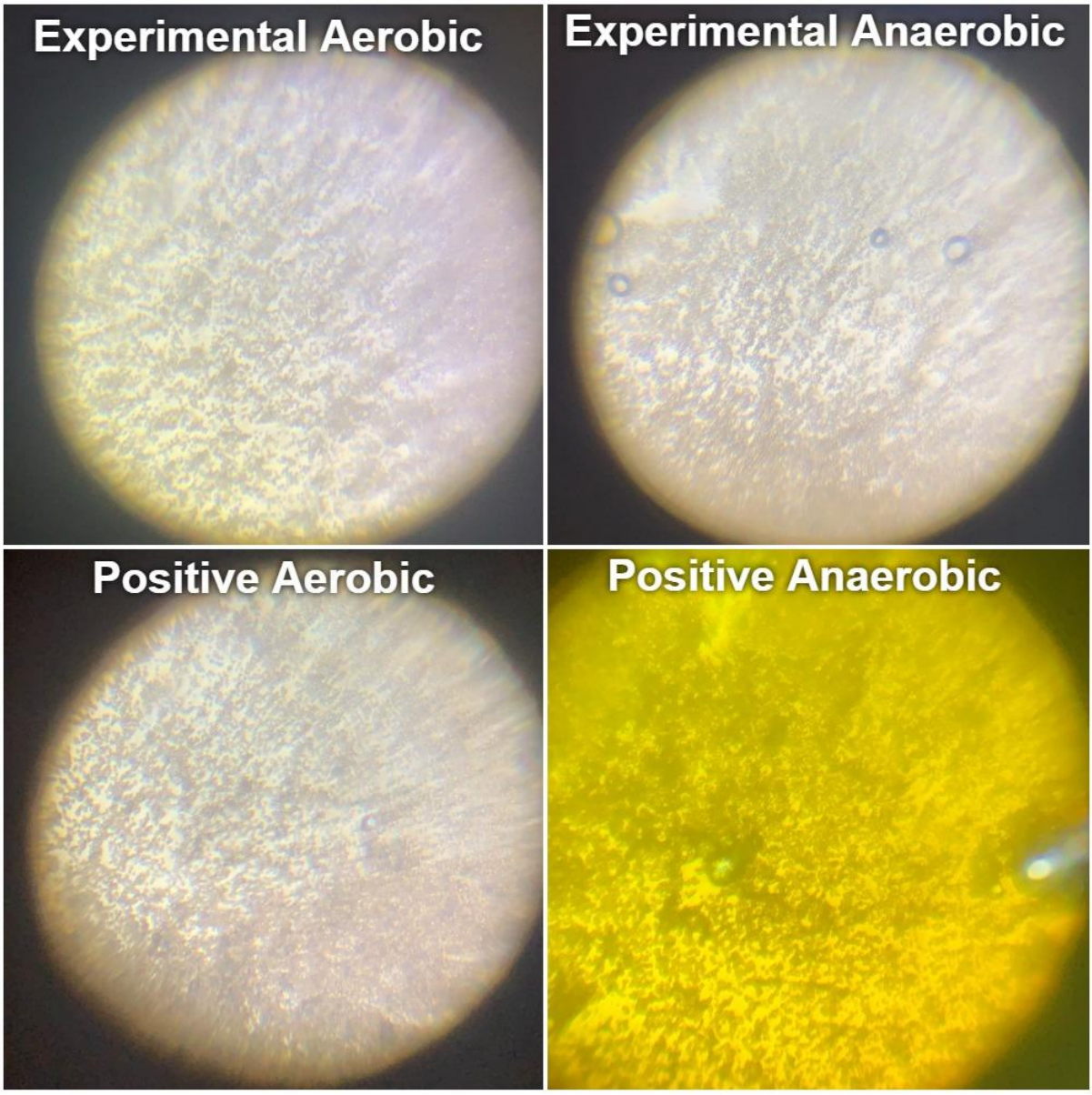


Figure 3. *The bottom right image's change in background color to complete yellow is due to excess iodine staining. Foreign molecules are detected in the center of that image in the form of a blackish yellow circle. Water molecules are also detected in the image experimental anaerobic respiring yeast culture on the center left and right side of the picture.*

Data Analysis

Throughout the experiment, carbon dioxide concentration was the dependent variable used to determine ascorbic acid's effects on S.cerevisae's respiration. Preliminary ANOVA hypothesis testing suggests that there is no interaction between the yeast's respiration and the different dosage factors of ascorbic acid. Table 3 supports this claim, as shown in the third column, as the p-value (0.719) is not within the area of the significance level (0.1). Graph 1 alongside the summary table, further supports the non-significance of the data—as for example, the point estimate mean for the aerobic respiring culture exposed to 0.5 mL of ascorbic acid in trial 1 can still deviate by 208.48—which falls within the interval of the positive aerobic control.

Experimental precision is left a bit to be desired, table 2 shows that the negative controls in each dosage factor may not always produce 0 ppm— despite the purpose of the negative control being to produce no data. Graph 2 also indicates that the individual CO_2 concentration for the positive respiring yeast cultures contained outliers.

Discussion

The results yielded results that failed to reject the null hypothesis—which asserts that the difference in numerical values in the respiration data has no relationship with ascorbic acid. One possible explanation for the failure to reject the null was the poor sample size this experiment used for the treatment groups. The lack of sampling variability, means that the results were dependent on factors found within the yeast cultures being measured. Alternatively and more likely to be the case, this null hypothesis truly reflected the effect of ascorbic acid when inserted into the yeast. The microscopic images reinforce this claim, as it provides little to no convincing evidence that cell lysis occurred in the experimental group despite hypothesizing earlier such an event might be plausible if respiration was inhibited. In

figure 3, the distribution of *S.cerevisae*'s presence is approximately identical to each other, figure 2 displays this phoenomanum too. Although the aerobic respiring experimental culture seems to have less yeast cells in its surroundings, the excess water bubbles found throughout the image and it being more prevalent compared to any of the other pictures suggests that the yeast cultures could have been swept away by the water bubbles to other parts of the microscope slides. Figure 1's yeast follows approximately the same cell count as the previous 2 figures would show. Furthermore, in some of the figures (e.g Figure 2 positive anaerobic and experimental anaerobic and Figure 1 experimental aerobic and positive aerobic), the clustered cells suggests that the yeasts under observation were specifically biofilm dispersed cells— given that both experimental and positive cultures are producing biofilms, this reinforces the connotation that cellular respiration and the mitochondria were not affected. However without an electron microscope, there is no visual evidence to prove whether these cells were truly intact as a result of the organic compound. The difference between *S.cerevisae*'s cellular respiration and the *C.albicans'* respiration is the latter's use of NADH complex I throughout the process. The change in outcome from this study's finding suggests that ascorbic acid actively denatures complex I—the effectiveness of ascorbic acid as a treatment is dependent on whether the organism in question is reliant on complex I for mitochondrial respiration.

Conclusion

The purpose of this experiment was to provide empirical evidence on the effectiveness of ascorbic acid as a possible treatment that targeted the cellular respiration of *S.cerevisiae*. It has been rationalized and predicted that based on the effectiveness of ascorbic acid treatment towards *C.albicans*, that the end result would have been homogenous, however this was proven false by the ANOVA testing, as the probability of obtaining such a sample under the pretense the null hypothesis was true is 71%— a value significantly higher

than the alpha threshold. In addition, there has been not only a lack of evidence that yeast cellular necrosis in any way has occurred, but also, conversely, the suspected presence of biofilm across the experimental groups further suggests that the mitochondria has not been impaired by the insertion of ascorbic acid into the culture medium.

Evaluation and Future Directions

As the experiment failed to reject the null hypothesis, exploration of ascorbic acid as a potential treatment for *S.cerevisiae* infections has no future direction, however there are nevertheless improvements that can be made to this experiment. The most serious error committed throughout the course of the entire experiment was the lack of subsequent trials done towards the experimental group and the consequence of this failure reverberates across the results and statistical calculations. This is best demonstrated by the margin of error calculated in the summary table, as a large deviation from the point estimate is present in almost every trial. This huge range deters the reliability of the experiment's results of *S.cerevisae*'s susceptibility to ascorbic acid. Furthermore, although there were enough trials to detect outliers in the positive control groups, for other cultures, the limitations of being bound to three trials prevented the computation of an interquartile range. This limited precision and skewed the final mean—as data inconsistencies could not be thrown away and justified as an anomaly. To circumvent this issue, the increase in the number of trials for future iterations of this experiment is highly recommended, which will not only lower the variability in the margin of error— providing more clarity on the statistical significance of the data, but also lower the probability that the failure to reject the null hypothesis is a case of type II error.

Despite the best efforts to prevent any extraneous variables from influencing the data through practicing biosafety regulations, there are still confounding variables that when accounted for— damages the reliability of the data. Glucose respiration was not the only

dependent variable being measured in this experiment, which is best observed in some of the negative controls having an increase in CO_2 concentration despite being grown in no culture medium. On the day of equipment disposal, the formation of molds on the petri dish is testimony that the petri dishes were contaminated with other microorganisms, and thus, the data gathered is possibly an overestimate of the CO_2 concentration produced if the medium only grew baker's yeast. This is a confounding variable brought forth by budgeting constraints— if this experiment was performed in a laminar chamber, the contamination of the petri dish by other microbes would be severely limited. Mold spores present may also be further circumvented by having this experiment be performed in a positive pressure chamber.

Although there is no future directions of using ascorbic acid as a possible treatment for *S.cerevisiae*, the data obtained from this experiment in relation to previous studies with yeast cells containing complex I suggests that there could be a correlation between ascorbic acid's ability to inhibit this coenzyme. Future research may confirm this prediction by conducting an experiment which measures complex I's function in the presence of ascorbic acid in varying microorganisms.

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